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(71) Applicant *(for all designated States except US)*: THE SCHOOL OF PHARMACY, UNIVERSITY OF LONDON [GB/GB]; 29/39 Brunswick Square, London WC1N 1AX (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : WARBURTON, Brian [GB/GB]; The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX (GB).

(74) Agent: JONES, Helen, Marjorie, Meredith; Gill Jennings & Every, Broadgate House, 7 Eldon Street, London EC2M 7LH (GB).

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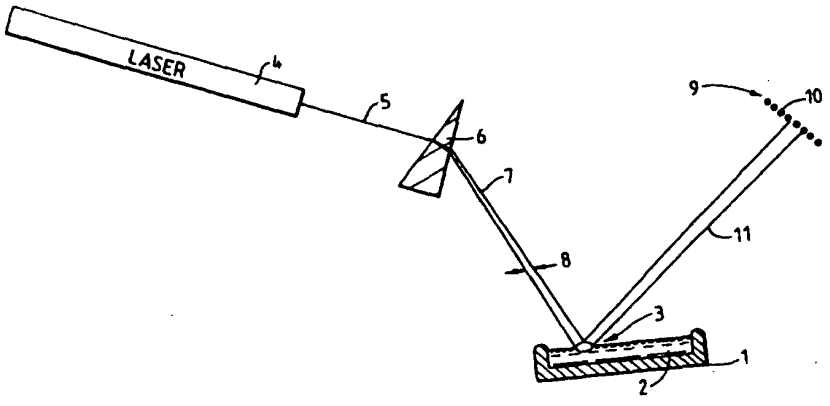

(57) Abstract

An optical or laser trapping device (also known as an optical tweezer) is used to trap a particle within a fluid. Usually the fluid is a liquid (2) and the particle (3) is suspended at the surface of the liquid. The particle is trapped in the optical tweezer and caused to move by an optical force created by displacing the beam (7), for instance by applying an electric field across a nicol prism (6) in the path of the beam. The motion of the particle under the applied optical force is observed, for instance by detecting light reflected from the particle using an array (10) of photodiodes. Preferably an oscillatory motion is applied to the beam and thence to the particle. The amplitude and/or frequency of the oscillation, as well as its phase relative to the phase of the laser beam motion, give information concerning the surface rheology, for instance the viscosity and elasticity, of the liquid. The apparatus is, for instance, used to observe clotting reactions in biological systems, for instance the clotting reaction of horseshoe crab amoebocyte upon contact with endotoxin.

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METHOD OF RHEOLOGICAL INVESTIGATION

The present invention relates to a method for investigating the rheology of a fluid using an optical trapping procedure of a small dielectric particle within the fluid. The optical trap is usually a single laser beam optical gradient trap. The method may be used to investigate the change of rheology of a liquid, for instance to monitor the course of clotting reactions, for instance reactions based on horse-shoe crab amoebocyte lysate.

Much useful information concerning fluids, especially liquids, can be obtained from investigating the rheology. It is possible to investigate the bulk rheology of the fluid and/or the surface rheology, which may be at the liquid-gas interface or at a liquid/liquid interface for a two-phase system comprising two immiscible liquids. Surface rheology investigations can provide much useful information concerning thin films of second components at the surface, for instance mono layers or multi layers, especially of surface active materials. Furthermore monitoring the change of bulk or surface rheology with time can provide much useful information concerning the kinetics of film formation, for instance films of biological materials especially proteins, materials useful in the formation of microcapsules, the formation and stability of emulsions, paint and other coatings and their drying/curing processes. A recent review of surface rheology investigation has been published in "Techniques in Rheological Measurement" ed. A A Collyer, Chapman and Hall, London, (1993) in chapter by B. Warburton.

One reaction of particular interest where monitoring the change of rheology is of interest is in clotting reactions in biochemical systems, for instance blood. In EP-A-0,325,874 an apparatus for monitoring the time for

coagulation of blood plasma is described. This consists of a cuvette having a base including a concave track within which a steel ball can roll. The apparatus comprises means for applying a magnetic field to act on the ball which can cause it to undergo oscillatory movement in the track. To measure the coagulation time, a sample of blood plasma is placed in the cuvette. A change in the period of oscillation of the ball, detected by optical means, provides information concerning the rate of coagulation of the blood.

Another biological clotting system of commercial interest is the clotting reaction of horse-shoe crab amoebocyte lysate. When this material is exposed to minute quantities of endotoxin the lysate increases in opacity and viscosity and may gel, depending on the concentration of endotoxin. Limulus amoebocyte lysate (LAL) is used in a test which can be used as a substitute for the pharmacopoeial rabbit pyrogen test, and can detect concentrations of endotoxin (pyrogen) as low as 0.5 ng/ml.

The basic constituents of LAL are proclotting enzyme activating factor, proclotting enzyme (inactive), coagulable protein and divalent cations. The activating factor is switched on in the presence of endotoxin, causing activation of the proclotting enzyme cascade series. The activated proclotting enzymes effect gelation in the LAL by cleaving the coagulable protein, the peptides formed therefrom being rendered able to react together via disulphide bonds of cystine units, to form a three-dimensional matrix which exhibits a gel-like structure.

When used to identify the presence of endotoxin, the end point can be determined by observing the clot. However existing techniques are crude, relying on the clots holding the walls of a tube when inverted giving a positive or a negative result. It is difficult to obtain quantitative information from the results and this can only be done by carrying ut several individual tests.

Turbidimetric methods which observe the change in turbidity at the end point rather than the change in viscosity have also been suggested, which are more quantitative and relatively successful.

A more sensitive technique is the use of chromogenic or fluorogenic synthetic substrates for the clotting enzymes, which compete for enzyme sites with the coagulable protein. When cleaved they release a product which is differently coloured or fluorescent from the starting material. The product can thus be identified and quantified. These chromogenic techniques are more sensitive and quantitative than the gel clot techniques.

A problem with all techniques based on optical observations is that coloured samples can interfere with results.

Nithiananthan, in his Ph.D. thesis "New Techniques for the Detection of the Limulus Amoebocyte Lysate End Point" University of London November 1991, describes a new technique for observing the surface rheology using an oscillating ring surface shear rheometer developed by Sherriff and Warburton (1974) Polymer, 15:253, using developments described by Kerr (1985) Ph.D. thesis, University of London and Barnes DMH (1988) Ph.D. thesis, University of London. In this technique a platinum Du Nouy ring (White Electrical Company, Malvern, Worcestershire) is placed in the plane of the sample surface, which is attached to the oscillating coil of a galvanometer. The ring is made to oscillate thereby communicating a surface shear stress. The amplitude ratio and phase angle ratio of the input and output wave forms are used to determine the components of the test material's surface shear modulus.

Although the technique is quantitative and sensitive, it is a time consuming technique and is unsuitable for or at least difficult to adapt to multiple sample testing. Furthermore placement of the ring requires a considerable degree of pr cision and patience, and maintaining an

undistorted ring positioned accurately is crucial and difficult to arrange in practice.

A controlled stress (bulk) rheometer was also tested by Nithiananthan but he found it had poor sensitivity and gave unsatisfactorily variable results.

A problem with the conventional procedures for determining the end point using optical measurements are that it is difficult to determine the end point where samples are coloured, opaque or viscous.

Lasers can be used to trap and manipulate electrically neutral particles. The use of optical trapping of small dielectric particles was first described by Ashkin in the early 70's (Ashkin, A., Phys. Rev. Lett. 24, 156 (1970) & 25, 1321 (1970)). The technique of optical trapping and some of the applications thereof are described by Steven Chu in Scientific American February 1992, 48-54.

Micron and sub-micron size transparent dielectric spheres have been trapped in optical gradient traps. Single beam gradient traps consist of a single strongly focused gaussian laser beam having gaussian transverse intensity profile. In such traps the basic scattering forces and gradient force component of radiation pressure are configured to give a point of stable equilibrium located close to the beam focus. Particles in the trap are confined transverse to the beam axis by the radial component of the gradient force, whilst they are confined in the axial direction by the beam focusing which creates the axial gradient force. Particles which can be trapped range from about 10 μm down to a few Å.

Ashkin and Dziedzic in Science, 235, 1517 (1987) describe the use of the "optical tweezers" for moving live bacteria whilst being viewed under a high resolution optical microscope, as well as the trapping of tobacco mozaic virus (TMV), polystyrene latex particles and silica particles. In Nature, 338, 514 (1989) Block et al describe the use of optical tweezers to determine the torsional compliance of flagella of bacteria. A tethered bacterial

cell was rotated about its tether by trapping the untethered end and moving it within the trap. The torque required to rotate the cells was determined, quantitated by calibrating the trap against Stoke's drag by measurements on a free cell. Since the cells normally spin when tethered, the power of the flagella motor can be determined.

Other applications for optical tweezers which have been described include the manipulation of chromosomes and other organelles within living cells, studies on muscle contraction at the molecular level, manipulating DNA molecules, eventually to examine the motion of enzymes along DNA, and movement of kinesin along microtubials, as well as observing sperm movement.

In US-A-3,710,279 Ashkin describes several embodiments of optical tweezer device and the application of the device to heating a tungsten particle to form a light source, act as a neutral particle gun and otherwise act as a high energy neutral particle accelerator, a gas isotope separation apparatus, a vaccuum deposition apparatus, a device for measuring the tensile strength of the particle and other applications, in all of which a particle is moved within a gas.

In Optics Letters, 16 1463 (1991) Sasaki et al describe the application of laser trapping (optical tweezer) in the field of biology and chemistry, as a means of organising functional materials, in order to arrange reactive particles for particles for interaction in a particular conformation. The particles which are manipulated are suspended in a liquid, for instance an alcohol or a glycol. The authors recognise that the particle flow velocity in the system is determined by the optical force acting on the particle, frictional forces between the particles and a plate against which they bear, and the viscous resistance by the surrounding liquid medium.

In JP-A-04-036637 (1992) latex particles are manipulated by an optical trap which acts to converge and agglutinate the particles.

The present invention utilises the optical tweezer to investigate the rheology to of a fluid.

A new method according to the present invention of investigating the rheology of a fluid involves introducing a dielectric particle into the fluid, trapping the particle in an optical trap and causing the particle to move in the fluid by applying an optical force to the particle and measuring the motion of the particle in the fluid.

The method may be used to investigate the surface rheology of the fluid, in which case the particle is trapped at and manipulated at the surface of the fluid. The fluid is usually a liquid, and the surface may therefore be the liquid/gas (usually liquid/air) interface or may be the interface between the liquid and another, immiscible liquid phase. Measuring surface rheology of a biological polymer-containing liquid is a particularly suitable use of the invention since such compouunds tend to have a degree of surface activity and thus to concentrate at the liquid/air interface. The surface rheology measurement may thus be more sensitive than a bulk rheology measurement.

Alternatively the method may be used to determine the bulk rheology of a liquid. In this instance the particle is trapped and manipulated within the bulk of the liquid.

The process is of great value for investigating the rheology of liquids which comprise dissolved or dispersed relatively high molecular weight materials, usually polymeric materials including biochemical polymers such as proteins, nucleic acids, lipids or polysaccharides or combinations of any of these as well as synthetic polymers. Where the surface rheology of a liquid is investigated the liquid may include in addition to or as an alternative to the polymeric materials, surface active materials, such as lipids, soaps, detergent or oth r surfactant materials etc.

The method of the invention is of particular value for monitoring a change in rheology of a fluid especially a liquid with time, for instance to monitor a clotting or gelling process. Such processes often involve the rheology of a liquid comprising dissolved protein, for instance the components of a blood clotting system. One example may be to monitor human blood clotting time, for instance as a diagnostic tool. For instance the method is of particular utility for monitoring the clotting process of a horseshoe crab amoebocyte lysate, especially in an assay using such lysate to determine the presence and/or level of endotoxin in a sample.

The method of the invention may also be used to monitor film formation, and may for instance be used to monitor formation of protein-containing films especially under various conditions of pH, temperature etc, of the drying and/or curing of paint and other surface coatings, for identifying substances which may be used as micro-encapsulation shells, as well as investigating the properties of materials to be used as emulsifiers, for instance in the pharmaceutical, agrochemical or food industries.

The invention is of particular value in a process for assaying a sample for the presence of endotoxin (pyrogen) using the amoebocyte lysate test, in which the process of the invention is used to monitor any clotting reactions taking place when the sample and the amoebocyte lysate are contacted in aqueous suspension. In a preferred embodiment of this process the rheology at the surface of the suspension, where the active protein components tend to concentrate, is monitored.

In the preferred application of the present invention, for monitoring the viscosity during the amoebocyte lysate test, the amoebocyte lysate used in the LAL test can be any of the lysates known to clot in the presence of gram-negative toxins, and are typified by the reagent which is commonly known as LAL. In mor detail, the reagents are

the amoebocyte lysate extracted from the horseshoe crab (species Limulidae, subclass Xiphosura of the Meristomato phylum) eg Tachypule gigus, Carcinoscorpius rotundicauda, and in particular preferably Tachypleus tridentatus and Limulus polyphemus. These crabs are used as live blood donors and after extraction of blood sample can be returned to the ocean unharmed. The lysate that is used in the invention is preferably a typical LAL or other amoebocyte lysate reagent as supplied commercially, and so the lysate generally includes the conventional additives that are present in such reagents, for instance buffering agents. The reagent may be a derivative of amoebocyte lysate, but must include at least the proclotting enzyme activating factor (with which endotoxin interacts), proclotting enzyme and coagulable protein. It may be supplied for use in dried stabilised form, for instance in freeze-dried form, and so is resuspended before use in water or aqueous solution (pyrogen-free). The lysate or extract may be made up at the regular concentration i.e. as used for the standard methods of clotting in a tube, but this technique may allow for more dilute levels to be used.

The sample which is assayed for the presence of endotoxin may for instance be a sample of body fluid, eg blood, urine, saliva, cerebrospinal fluid or a swab from the body, or may be water, for instance drinking water, or may be a food or pharmaceutical product. The sample may be used as such in the test, or may be pretreated to remove possible inhibitory factors before testing or may be plated out and allowed to culture for a period before being tested. The potentially endotoxin-containing material will usually be provided in the form of an aqueous suspension before being contacted with the amoebocyte lysate and that aqueous suspension may contain buffers, salts, metal ions, sequestering agents, coloured products, eg iron dextran; opaque liquids, eg intralipids; and viscous liquids, eg intrathecal injection fluids. The process may be used for

instance as an in-line quality control monitor in food, drink or pharmaceutical production processes.

The rheology of the fluid is investigated by first trapping an inert particle within a fluid or on the surface of a liquid the laser beam, and then causing the trapped particle to move within the suspension using the laser beam, and analysing the particle's motion as a function of the suspension rheology. The rheology, eg the elasticity and/or viscosity, can then be used to give a qualitative and/or quantitative result, for instance where the progress of a biochemical or chemical reaction is being investigated or the presence of an analyte (eg endotoxin) is to be determined.

The rheology to be measured may be the rheology of the bulk fluid, or of the surface of a liquid, for instance of a cast film of the liquid and the property measured may be the viscosity and/or elasticity. The rheology may be measured at any time during the course of a reaction, and therefore can give information on changing rheology with time. Usually the change in rheology will be an increase in viscosity but could also be a decrease in viscosity occur during a reaction under test and/or a change in the elasticity. Preferably the particle is trapped at the surface of the liquid in order that it is more easily located and trapped by the instrument. The process of the invention thus preferably is used to investigate the surface rheology of the suspension.

The inert particle to be trapped by the optical tweezers are selected from materials according to the particular type of rheology to be measured eg bulk viscosity, surface viscosity etc.

When the bulk rheology is to be investigated the inert particles must have a density substantially equal to that of the fluid in which they are suspended. Where the fluid is a liquid, eg conveniently an aqueous liquid the overall density of the particles is approximately 1 eg in the range 0.95 up to 1.1, preferably less than 1.05 g/ml. Suitable

materials include polystyrene, silica, glass, polycarbonate. The density of the particles may be adjusted by forming them as hollow beads if the materials are a high density. The relative density will vary or can be adjusted by a change in temperature.

When the surface rheology is to be investigated the inert particles must necessarily have a density which is less than that of the liquid. Where the liquid is aqueous, therefore the density must be less than 1 for instance less than 0.95. Suitable materials include polypropylene and other polyolefins, eg polyethylene or polybutadiene.

Further, small hollow capsules, again having a density less than that of the liquid but formed from materials having a density, and therefore rising to the surface of the suspension, can also be used for the surface viscosity measurement.

Alternatively certain hydrophobic particles may be used, which as a result of surface tension techniques become trapped in the surface of the liquid.

The particles used in the invention generally require to be of regular shape and size. They are preferably substantially spherical, although in some instances it may be convenient for them to be rod shaped or cubic.

Regular sized and shaped polyolefin particles are made for instance by laser trimming. Various methods for making microcapsules, especially spherical ones, are known in the art, for instance as described in GB-A-2,009,698 from synthetic and/or natural polymers.

There may be one or several particles suspended in a suspension under test. Where there are more than one particle it may be more easy to locate and trap a single particle in the beam. Techniques are available for automatic (computer-controlled) identification of particles optically, such as are used in cell identification especially for identifying abnormal cells.

The size of the inert particles which can be used ranges from 0.01 to 10 μm. Preferably the size of the

inert particles is comprised in the range 0.1-5 μm. In the method the volume of sample used may be very small and one of the advantages of the technique of the present invention is the scale of the test volume. For instance the fluid volume used in the method is less than 10 ml, preferably less than 1 ml for instance about 100 μl.

In the method it is generally necessary to control the temperature of the sample under test in order to ensure there are no superimposed rheology changes by variations in temperature. The apparatus used for the method thus includes temperature control means. Since the volume of fluid under test is so low the temperature can be adjusted quickly to the desired temperature of the test.

Several methods of investigating the rheology, for instance by measuring the viscosity or a change from a liquid to an elastic gel of the suspension can be used. One method includes causing the particle trapped within the laser beam to describe oscillatory motion for instance by moving the particle in a substantially straight path in the suspension, preferably under sinusoidal motion, to facilitate analysis of the results. The amplitude of the particle's motion, is preferably small relative to the particle size, that is it is preferably less than 50% of the diameter of the particle, more preferably less than 20% of the particle diameter. As the viscosity of the test suspension increases, the velocity of the particle, and therefore its frequency of oscillation, decreases with the same force being applied or alternatively, if frequency remains the same, the amplitude will decrease and there will be a change in the phase lag if and when liquid changes to a gel, an elastic material. This can be used to produce displacement-time or phase-time graphs at different times throughout the course of a continuing reaction. These graphs can then be super-imposed on top of one another to show the effect of changing rheology with time as th reaction proceeds.

By calibrating the graphs obtained against those from suspensions of known rheology, for instance viscosity, the absolute viscosity and elasticity of the test sample can be elucidated. Alternatively the absolute viscosity could be determined by calculation from a knowledge of the particle's properties.

Another way of using the optical tweezers to investigate the rheology of the suspension is to cause the inert particle to move a pre-determined distance through the suspension, and to measure the time taken for it to cover this distance, so obtaining the velocity of the particle. A non-spherical particle could be made to rotate in the fluid. In either case as the particle moves a Stokes' drag acts in a direction against the motion; by equating the optical trapping force used with the Stokes' drag, and substituting in the measured velocity, the viscosity of the suspension can be calculated. Alternatively, velocity can be measured throughout the course of the reaction and velocity time graphs plotted. These can then be calibrated against velocity-time graphs obtained from suspensions of known viscosity to give the unknown viscosity.

Since these methods allow the measurement of rheology as a function of time they can be used to determine information on the profile of a reaction under test, including information on rate of reaction.

By comparing the results of the rheology determination against calibrating standards, the results can be expressed in terms of the absolute viscosity and/or elasticity of the solutions under test. Alternatively or additionally the results may be expressed in terms of some other property. For instance where the process is used to investigate the presence or level of endotoxin in a sample by the amoebocyte lysate reaction, the results may be expressed in terms of the level of endotoxin in the original sample. This may be done, for instance, by comparing the results

with calibration tests carried out using samples with known quantities of endotoxin, determined by other techniques.

In the invention the optical tweezer generally comprises a single-beam laser gradient trap, for instance of the type described in the literature cited above especially in US-A-3,710,279. The particle can be visualised optionally by optical microscope means using a separate source of incident light. Alternatively, and preferably, since it avoids extra components the laser light scattered from the particle surface, for instance at one position at 90° to the incident laser light, may be detected and its exact location and phase recorded. A comparison of the phase angle between the input disturbance light and the movement output at predetermined times gives an indication of the type of rheology and monitoring a change can indicate a change from Newtonian characteristics to other, for instance more elastic, gel-like characteristics which may report the displacement/time characteristics of the trapped particle with the aid of a human operator or fully automatically. The illumination for the optical microscope may be directed at the sample under observation in the same direction as the laser beam or from a different direction.

In figure 1 there is shown a diagrammatic representation of apparatus used in the invention.

The figure illustrates a vessel 1 for containing the liquid sample 2 under test. Suspended at the surface of the liquid is a particle 3, which has a specific gravity of less than that of the liquid 2. The vessel 1 may be mounted at one station on a multi-station apparatus, for instance provided on a turn-table, which is provided with means for its rotation to allow successive stations to be positioned for being subjected to the optical tweezer.

There is provided a laser 4, which emits a beam 5 which is directed through a nicol prism, 6. The nicol prism includes means f r providing an electric field at right angles to the beam of light, the strength of which

can be varied. Under the varying field, the polar molecules within the prism will bend the light by differing angles, thereby displacing the emitted beam 7, for instance by an amount varying between the extremes shown at 8 (not to scale). The motion of the emitted light is preferably sinusoidal in nature.

The detector, 9, consists of an array 10 of, for instance, photodiodes which can detect light, 11, scattered from a particle 3 in the beam 7. The photodiodes may be connected to processes, which in turn may control lenses (not shown) to control the direction of the laser beam on the search and detection of a particle in the sample container. The photodiode and the processor means also allow the phase of the scattered light to be determined and the processor means can provide a comparison with the phase angle of the incident light. The photodiodes allow the position of the particle to be monitored also. The processor is also connected to the nicol prism to receive information concerning the frequency and amplitude of the refracted laser light.

In the apparatus an alternating current applies an electric field across the nicol prism. Where the current has a sinusoidal wave form, the light displacement x is given by the formula $x = x_0 \sin 2\pi ft$, where f is the frequency and t is the time. For small displacements the Force exerted will be proportional to the displacement, x and virtually in phase with x, so that $F = kx = kx_0 \sin 2\pi ft$.

At the detector, the received light may contain a mixture of

a) source signal expressed by the term $f_1 kx_0 \sin 2\pi ft$ and

b) a phase displaced response, represented by $f_2 kx_0 \sin (2\pi ft + \Phi)$.

By determining these two components at given times after the start of the test, information concerning the rhe logy of the test sample can b obtain d.

The following example illustrates one application of the present invention:

Example

0.5 mls of test liquid is thoroughly mixed with 0.1 ml of LAL reagent made up in pyrogen-free water to a suitable dilution. A small number of inert beads which have a specific gravity less than that of the LAL reagent are dispersed into the mixture. The mixture is then placed in a pre-thermostatic well which is maintained at 37°C (the optimum temperature for the LAL reagent). Under computer guidance one bead is selected and optically trapped in the laser beam.

Application of a small sinusoidal displacement of the light beam not greater than 20% of the bead diameter then proceeds by applying an alternating current across the nicol prism of the apparatus illustrated in figure 1. The response at the detector array is analysed using fast fourier transform algorithm (FFT).

Initially the phase between the input wave form and the output wave form would be theoretically near to a phase lag of $\pi/_2$ radians or 90°. This is because the components of the test in the surface would comprise a Newtonian surface liquid. However if the test proved positive as time elapsed, the surface film would be expected to form a gel network. During this process the film would become more elastic and the phase angle would be expected to decrease and approach 0, the theoretical limit for a purely elastic film. Useful information could also accrue from the amplitude ratio of the disturbance input and the movement output.

CLAIMS

1. A method of investigating the rheology of a fluid in which a dielectric particle is introduced into the fluid, the particle is trapped in an optical trap and is caused to move in the fluid by applying an optical force to the particle, and the motion of the particle is measured.

2. A method according to claim 1 in which the surface rheology of the fluid is measured and in which the particle is trapped at and manipulated at the surface of the fluid.

3. A method according to claim 2 in which the fluid is a liquid and the surface is the liquid/air interface.

4. A method according to claim 1 in which the bulk rheology of the liquid is investigated and in which the particle is trapped and manipulated within the bulk of the liquid.

5. A method according to any preceding claim in which a change in the rheology of the fluid is determined by carrying out the method at least two times over a predetermined period of time.

6. A method according to claim 5 in which a clotting or gelling process is monitored.

7. A method according to claim 6 in which the clotting of a composition comprising horseshoe crab amoebocyte lysate and a sample potentially containing endotoxin is measured.

8. A method according to any preceding claim in which the rheological property measured is viscosity or elasticity.

9. A method according to any preceding claim in which the particle is formed of a material selected from polystyrene, polyolefins, silica, glass and polycarbonate.

10. A method according to any preceding claim in which the size of the particle is in the range 0.01 to 10 μm, preferably in the range 0.1 to 5 μm.

11. A method according to any preceding claim in which the force applied to the particle causes it to describe oscillatory motion, preferably sinusoidal.

12. A method according to claim 11 in which the amplitude of oscillation is less than 50% of the diameter of the

particle, more preferably less than 20% of the particle diameter.

13. A method according to any preceding claim in which the optical trap comprises a single-beam laser gradient trap.

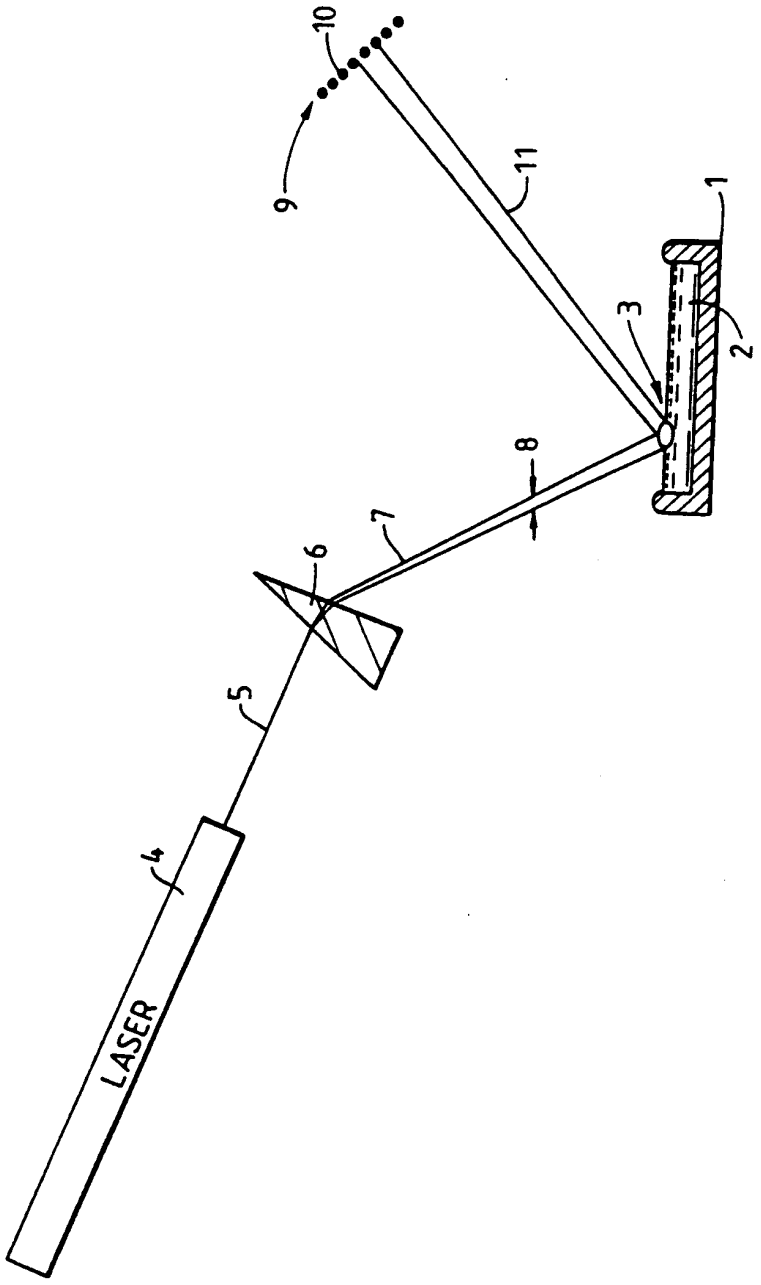
14. A method according to any preceding claim in which the motion of the particle is measured by detecting light scattered from the particles surface derived from the light used for the optical trap and optical force.

15. Apparatus comprising a source of incident light, a vessel for containing a fluid under investigation, a particle in the fluid within the vessel, the incident light source being arranged so as to be able to optically trap the said particle in a beam of incident light produced by the source, means for moving the incident light beam first to apply an optical force to the trapped particle and a detector for measuring the motion of the particle when subjected to the optical force.

16. Apparatus according to claim 15 in which the apparatus includes means for conferring upon the incident light beam a oscillatory motion, preferably of a sinusoidal nature.

17. Apparatus according to claim 16 in which the amplitude of oscillation at the particle is less than 50% of the particle diameter, preferably less than 20% of the particle diameter.

18. Apparatus according to any of claims 15-17, in which the detector comprises an array of photodiodes arranged for detection of incident light which has been scattered by the particle.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LASER

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 93/02017

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 G01N11/10 H05H3/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 G01N H05H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 325 874 (SERBIO) 2 August 1989 see abstract ---	1,4-6,8
A	POLYMER vol. 15 , April 1974 pages 253 - 254 SHERRIFF ET AL. 'Measurement of dynamic rheological properties using the principle of externally shifted and restored resonance' cited in the application see page 253, left column ---	1-3,5,6, 8,11
A	US,A,3 710 279 (ASHKIN) 9 January 1973 see column 1, line 40 - column 3, line 54 see column 17, line 25 - column 18, line 13 --- -/--	1,9,10, 15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
17 December 1993	22.12.93

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Bindon, C

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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	OPTICS LETTERS vol. 16, no. 19 , 1 October 1991 , WASHINGTON US pages 1463 - 1465 SASAKI ET AL. 'Pattern formation and flow control of fine particles by laser-scanning micromanipulation' see page 1464, right column, line 3 - page 1465, right column, line 8 ---	1,9,10, 13,15
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INTERNATIONAL SEARCH REPORT

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International Application No
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